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REGULAR ARTICLE

Freeze-dried whole plasma: Evaluating sucrose, trehalose, sorbitol, mannitol and glycine as stabilizers

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Abstract Several groups report stability results for freeze-dried whole plasma intended for use as a transfusion product [Hellstern P, Sachse H, Schwinn H, Oberfrank K. Manufacture and in vitro characterization of a solvent/detergent-treated human plasma. *Vox Sang* 1992;**63:**178—185; Trobisch H. Results of a quality-control study of lyophilized pooled plasmas which have been virally inactivated using a solvent detergent method (modified Horowitz procedure). *Beitr Infusionsther* 1991;**28:**92—109; Hugler P, Trobish H, Neuman H, Moller, Sirtl C, Derdak M, Laubenthal H. Quality control of three different conventional fresh-frozen plasma preparations and one new virus-inactivated lyophilized pooled plasma preparation. *Klin Wochenschr* 1991;**69:**157—161; Krutvacho T, Chuansumrit A, Isarangkura P, Pintadit P, Hathirat P, Chiewsilp P. Response of hemophilia with bleeding to fresh dry plasma. *Southeast Asian J Trop Med Public Health* 1993;**24:**169—173; Chuansumrit A, Krasaesub S, Angchaisuksiri P, Hathirat P, Isarangkura P. Survival analysis of patients with haemophilia at the International Haemophilia Training Centre, Bangkok, Thailand. *Haemophilia* 2004;**10:**542—549]. Plasma coagulation properties are substantially impaired in these freeze-dried plasmas, while pH levels are close to alkaline.

In this work, plasma supplemented with 60 mM sucrose, trehalose, mannitol, sorbitol or glycine was freeze-dried. The samples were subjected to forced degradation at 40 °C for 10 days in order to quickly evaluate the effectiveness of the different stabilizers. Initial PT, APTT and TT values were 14.4 ± 0.5 s, 31.4 ± 1.5 s and 18.3 ± 0.6 s, respectively. At the end of the degradation period, PT, APTT and TT

Abbreviations: FFP, fresh frozen plasma; SD, solvent/detergent; PT, prothrombin time; APTT, activated partial thromboplastin time; TT, thrombin time; DSC,differential scanning calorimetry; vWf, Von Willenbrand factor.

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were substantially prolonged, and were 19.1 ± 0. 5s, 43.1 ± 0.6s and 26.1 ± 1.0s, respectively. In the presence of glycine, at the end of the degradation period, PT, APTT and TT values remained close to the initial values and were 15.5 ± 0. 4s, 35.7 ± 0.9s and 19.4 ± 0.2s, respectively. Percent activities of the coagulation factors V, VII, VIII, IX, X and the coagulation inhibitors protein C, protein S and antithrombin III were recorded. Factors V and VIII were most prone to degradation. Factor V and VIII activities, in control plasma, were approx. 44 ± 3.5% and 58 ± 2.3%, at the end of storage. In contrast, much higher factor V and VIII activities were maintained in the lyophilized glycine-supplemented plasma: approx. 60 ± 3.5% and 74 ± 7.0%, correspondingly. The most stable protein was protein C, which showed no signs of degradation under the testing conditions of this study. All tested stabilizers provided protection. Glycine, however, outperformed all tested polyols, providing superior preservation of plasma clotting properties. Thermograms of 60mM glycine in water and 60mM glycine in plasma show that, in the presence of plasma, glycine does not crystallize. The process of freeze-drying caused a complete loss of plasma pCO_2 (gas) and a substantial increase in plasma pH. Citric acid was found to be a suitable pH adjuster for lyophilized/rehydrated plasma.

Introduction

Fresh frozen plasma (FFP) is essential for the clinical management of coagulopathies associated with combat trauma. However, the frozen formulation has three major limitations: (a) FFP must be kept frozen at −18 °C or below; (b) the number of FFP units transshipped is restricted by the dry ice (CO_2(s)) limitations of air transport; (c) the thawing time of intact plasma units is 30 to 40 min.

Clearly, the limitations of the frozen formulation reduce plasma availability in the field, or in rural locations, for both logistic and practical laboratory reasons. A freeze-dried formulation, alternatively, could remedy the storage and shipment problem by converting liquid plasma into a lightweight, solid product that is stable at ambient temperature. Currently, the advantage of using freeze-dried plasma vs. frozen plasma has been mostly recognized in developing countries [4,5]. There are limitations in facilities for preparation, transportation and storage of frozen blood products in most of these countries. Therefore, freeze-dried products with less storage and transportation requirements are preferred.

Pooled plasma was lyophilized for the first time during World War II. However, it was discovered that the process of freeze-drying does not kill viruses in plasma. In addition, the use of plasma from large pools carried an unacceptable risk of transmitting pathogens [6]. Therefore, the production of freeze-dried plasma was abandoned.

Several methods for pathogen inactivation in plasma are now at different stages of development. Such methods are based on: solvent/detergent treatment [7]; utilization of vitamin B2, riboflavin and light [8]; application of psoralens and UV light [9]. The current endeavor is to freeze-dry pathogen-inactivated plasma products. These products will guarantee both unconstrained plasma availability and safety. Several groups report stability results for freeze-dried, pathogen-inactivated, solvent/detergent (SD)-treated plasma products. Hellstern et al. [1] describe the production of freeze-dried and deep-frozen batches of SD plasma, and characterize the product in vitro [1]. Clotting factors activities were found to decrease more markedly in the freeze-dried plasmas than in the deep-frozen batches [1]. Storage stability data at ambient temperature are not reported in this study [1]. The German Red Cross introduced a freeze-dried pathogen-inactivated SD plasma product in 1990. The product was examined to determine whether the quality was comparable to standard preparations. Several publications report the results of this study. It was found that freeze-dried SD plasma did not fulfill basic requirements. Shortly, the time required to reconstitute the lyophilized product was too long. The resultant pH values of the lyophilized/reconstituted plasma were close to the alkaline range; thus, considerable changes in blood gas and electrolyte levels were to be expected in the recipient [2]. In a separate study, the quality of three conventional fresh-frozen plasma preparations and one freeze-dried SD plasma preparation were compared [3]. Coagulation activity was significantly reduced in the freeze-dried SD plasma [3]. Storage stability data at ambient temperature are not reported in these studies [2,3]. In Thailand, freeze-dried plasma has been used by hemophilia patients as